Rapid Publications

Preferential Lysis of Pancreatic B-Cells by Islet Cell Surface Antibodies

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SUMMARY

Sera from patients with insulin-dependent diabetes mellitus (IDDM) containing islet cell surface antibodies (ICSA) were studied for their capacity to lyse cultured rat islet cells. The uptake of ethidium bromide was used to identify lysed cells and immunofluorescent staining with antisera to insulin, glucagon, somatostatin, or pancreatic polypeptide was used to identify the different islet cell types (B-, A-, D-, and PP-cells, respectively). Our experiments showed that in the presence of complement, sera containing ICSA lysed 81% of the B-cells, but 10% or less of the A-, D-, and PP-cells. Normal control sera resulted in lysis of less than 4% of each of the islet cell types. The demonstration that ICSA are preferentially lytic for B-cells may be important in defining the role of these autoantibodies in the pathogenesis of IDDM, particularly since the B-cell mass in diabetics is markedly reduced relative to the other islet cell types. DIABETES 31: 459–462, May 1982.

Insulin-dependent diabetes mellitus (IDDM) is characterized by a destruction of insulin-producing pancreatic B-cells.[1] Although at least three other islet cell types (A, D, and PP) are present in the islets of Langerhans, quantitative morphometric analyses of islets from patients with IDDM, as well as from experimental diabetic animals, indicate that these cell types are present in an increased proportion relative to the markedly reduced B-cell mass.[2–4] Non-species-specific but organ-specific antibodies directed against surface determinants of rat islet cells (islet cell surface antibodies or ICSA) have been detected in the serum of patients with IDDM.[5] We have shown that ICSA-containing serum, in the presence of complement, was cytotoxic to rat B-cells grown in monolayer culture.[6] This was determined by a double-label fluorescence technique in which lysed cells were identified by the uptake of ethidium bromide (seen as cells with orange nuclei) and B-cells were simultaneously identified by staining with fluorescein isothiocyanate-conjugated insulin antiserum (seen as cells with green cytoplasma). In the present study, we have modified this technique using antisera to the other islet hormones (glucagon, somatostatin and pancreatic polypeptide) to determine whether ICSA were capable of lysing A-, D-, and PP-cells, respectively. Data presented here indicate a preferential lysis of B-cells by ICSA.

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MATERIALS AND METHODS

Control sera were obtained from individuals (age range 2–10 yr) with no family history of diabetes. ICSA-containing sera were obtained from patients with IDDM (age range 1–12 yr), all of whom had at least one episode of ketoacidosis and had been treated with insulin. Sera were screened for ICSA by the ^{51}Cr release assay as previously described.[6] Twelve highly lytic sera from patients with IDDM (^{51}Cr-release: 47.2 ± 5.0%; range 40.2–57.3%) and ten sera from normal controls (^{51}Cr-release: 10.9 ± 4.8%; range 2.4–16.5%) were used in the present experiments.

To evaluate cell lysis, newborn rat islet cell monolayers were grown on glass coverslips,[7] then incubated at 37°C for 30 min with a 1:2 dilution of heat-inactivated patient serum. After rinsing in Dulbecco's phosphate buffered saline (PBS), the cultures were incubated at 37°C for 20 min with a 1:12 dilution of rabbit complement. Following a 10-min incubation with ethidium bromide (1 μg/ml PBS), the monolayers were fixed in Bouin's solution. Cultures were incubated at 4°C for 16 h with either guinea pig anti-porcine insulin serum (at a dilution of 1:1,280; from Dr. Peter F. Wright), rabbit anti-porcine glucagon (at a dilution of 1:60; from Cambridge Nuclear, Billerica, Massachusetts), rabbit anti-somatostatin (at a dilution of 1:40; from Immunonuclear, Minneapolis, Minnesota), or rabbit anti-bovine pancreatic polypeptide (at a dilution of 1:80; from Dr. R. E. Chance, Lilly Research Laboratories, Indianapolis, Indiana). Fluorescein isothiocyanate-conjugated rabbit anti-

guinea pig IgG or goat anti-rabbit IgG (Miles Biochemicals, Elkhart, Indiana) was used as the second antibody. Stained preparations were observed and photographed with fluorescein or rhodamine filter systems.[6]

The approximate percentages of B-, A-, D-, and PP-cells in the cultures, as determined by the immunofluorescent staining procedure described above, were 65, 20, 5, and 10, respectively.

RESULTS

The lytic effect of ICSA-containing sera on the different islet cell types is illustrated in Figure 1. When the cultures were viewed with fluorescein filters the cytoplasma of the various hormone-containing cells appeared green (column A). When the same fields were examined with rhodamine filters (column B), the nuclei and to a lesser extent the cytoplasma of lysed cells appeared orange. By double exposure photography (column C), the lysed hormone-containing cells appeared yellow (the result of superimposition of the green and orange fluorochromes); nonviable non-hormone-containing cells appeared orange, and viable hormone-containing cells appeared green. Row 1 demonstrates that ICSA were highly lytic for B-cells. On the other hand, rows 2, 3, and 4 show that ICSA were only minimally lytic for A-, D-, and PP-cells, respectively. In the presence of normal serum, minimal lysis was seen for B-cells (row 5) as well as for the other islet cell types (not shown). Lysis of rat fibroblasts (e.g., row 5, cells in the periphery of the field) was observed with all sera irrespective of the presence of ICSA, but could be removed by absorption.[6] No lysis of islet cells and fibroblasts was observed when PBS was substituted for patient serum or when heat-inactivated complement was used.

The percentage of each cell type lysed by sera from 12 patients with IDDM and 10 normal controls is given in Table 1. ICSA-containing sera were highly lytic for B-cells (81%). In contrast, 10%, 4%, and 7.6%, respectively, of the A-, D-, and PP-cells were lysed. Control sera typically lysed $<4\%$ of each islet cell type.

DISCUSSION

While the double-label fluorescence technique appears to be a valuable tool for identifying lysed cells in a mixed cell population, some precautions should be emphasized. For example, when cells are viewed with fluoresceine filters, the nuclei of lysed non-hormone-containing cells sometimes appeared faintly green due to "bleed through" of ethidium bromide fluorescence. Without careful examination, as to the intracellular location of the fluorescence, these cells could be incorrectly scored as lysed hormone-containing cells. Also, extensive lysis, as would be expected with prolonged incubation with complement, may result in significant degranulation of hormone-containing cells. This would lead to underestimation of lysed hormone-containing cells. However, under the conditions described above (i.e., short incubation with a relatively high dilution of complement), this was not apparent since the percentages of each cell type as determined by immunofluorescent staining, in ICSA/complement-treated and control cultures were similar (data not shown).

It is also important to note that the ICSA-containing sera used above were selected on the basis of their high lytic activity in a ^{51}Cr release assay[6] to maximize the chances of detecting other associated but minor antibodies. Therefore, while high percentages of lysis for B-cells were frequently found in serum samples from patients with IDDM, lower percentages (40–50%) were not uncommon. In this regard, the low degree of cytotoxicity for A-, D-, and PP-cells in ICSA-containing sera as compared with control sera (see Table 1) may indicate a weak cross reactivity between primarily B-cell specific antibodies and these cell types or low levels of other antibodies. Alternately, the increased B-cell destruction in ICSA/complement-treated cultures may nonspecifically damage adjacent cells.

Evidence for a humoral immune component in the pathogenesis of IDDM has been accumulating in recent years. Islet cell antibodies (ICA), detected in the serum of patients with IDDM appear to be directed against cytoplasmic components of all islet cells.[8] These characteristics suggest that ICA may develop secondarily as a response to islet cell damage.[9] On the other hand, the lytic properties of ICSA are consistent with a primary mediator of immune damage.

The demonstration here that ICSA are preferentially lytic for the insulin-containing B-cells may be one of the explanations for the selected loss of B-cells in patients with IDDM. However, what actually triggers the production of ICSA and the role that it plays in the pathogenesis of IDDM remains unclear. One possibility is that single or multiple environmental insults (e.g., viruses and/or chemicals) injure the islets and stimulate the production of autoantibodies.[10,11] Alternately, autoantibodies may result from an immunoregulatory defect in individuals with a genetic predisposition (i.e., HLA-linked). Our recent report of ICSA in 25% of nondiabetic first degree relatives of diabetic probands suggests that the mere presence of ICSA may not be sufficient to produce the diabetic state.[6] This finding may be a reflection of the complexity of etiologic factors in the development of IDDM which likely is further modified by the ability of B-cells to recover from these insults.

While it is assumed that ICSA cross-reacts with antigens present on the surface of both human and rat B-cells, the capacity of ICSA to lyse human B-cells has not yet been demonstrated. This experiment has been difficult to perform because of the technical problems involved in obtaining sufficient numbers of viable human islet cells. The demonstration here that the double-label fluorescence technique can be used to identify a small number of hormone-contain-

TABLE 1
Lysis of islet cell types by islet cell surface antibodies

Islet cell type	% Cells lysed*	
	ICSA positive	Control
B	81 ± 8.9	3.1 ± 1.8
A	10 ± 3.8	3.2 ± 1.7
D	4.0 ± 2.2	2.4 ± 1.7
PP	7.6 ± 3.3	3.4 ± 1.8

* Each of twelve ICSA-containing serum samples and ten normal controls were tested for lytic acitivity against each islet cell type as described in METHODS. By rapid back and forth switching between fluorescein filters (to identify the cell type) and rhodamine filters (to identify dead cells), the extent of lysis was quantitated. The percentage of lysis (mean ± SD) was determined by counting 200 cells for each cell type per serum sample. Similar results were obtained by counting lysed cells on double exposure photographs.

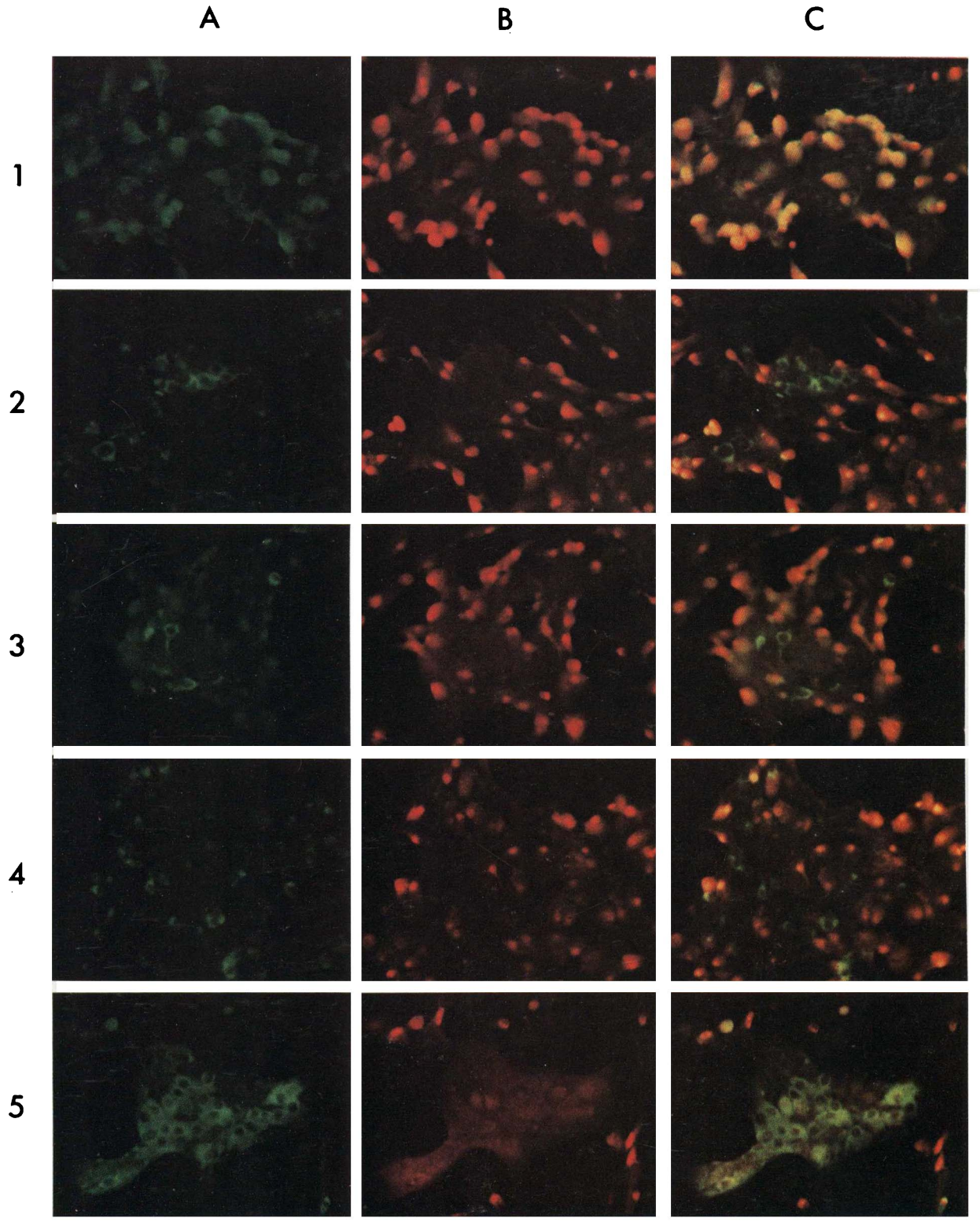

FIGURE 1. Lysis of islet cell types by islet cell surface antibodies. Monolayers of rat islet cells were incubated with ICSA-containing serum (rows 1–4) or normal serum (row 5). After successive incubations with complement and ethidium bromide, the cells were fixed in Bouin's solution and stained, using indirect immunofluorescence techniques for insulin (rows 1 and 5), glucagon (row 2), somatostatin (row 3), and pancreatic polypeptide (row 4). When viewed with fluorescein filters (column A), hormone-containing cells appeared green. When the same fields were viewed with rhodamine filters (column B), lysed cells (especially the nuclei) appeared orange. By double exposure photography (column C), lysed hormone-containing cells appeared yellow, nonlysed hormone-containing cells green, and lysed non-hormone-containing cells orange (magnification ×370).

ing cells in a mixed cell population suggests that this technique may prove useful in evaluating the capacity of ICSA to lyse specific populations of human islet cells.

Finally, the specificity of ICSA suggests its potential application in the purification of B-cells by flow cytometry[12] or affinity techniques.[13]

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Note added in proof. After submission of this manuscript, P. Sai and colleagues published a report on the effects of serum containing complement-fixing islet cell antibodies on in vitro insulin and glucagon release by mouse islets (Diabetes *30*:1051–57, 1981). Their finding of suppressed insulin release but not glucagon release also suggests that these antibodies may contribute to selective B-cell damage in IDDM.

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